Interaction between CYP1A1 Polymorphic Variants and Dietary Exposures Influencing Ovarian Cancer Risk

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Abstract
Aromatic hydrocarbon hydroxylase (CYP1A1) is involved in the metabolism of many substrates and the subject of cancer studies. This study examined the association between two polymorphic variants of CYP1A1 and ovarian cancer risk.

The frequencies of the Msp1 and Ile/Val variants of CYP1A1 were determined in 445 ovarian cancer cases and 472 general population controls in New England. Overall relative risks were calculated as well as those within subgroups of various exposures.

There was no increased risk for ovarian cancer associated with possession of either the Msp1 or Ile/Val polymorphism of CYP1A1. Elevated risk for ovarian cancer was found in those who carried an Ile/Val variant and who consumed more than median levels of caffeine (risk ratio = 2.69; 95% confidence interval, 1.18–6.18). No variation by histological type of ovarian cancer was observed.

Significant interaction may exist between polymorphic variants of CYP1A1 and caffeine that could explain weak or inconsistent associations between caffeine and ovarian cancer when genotype has not been considered.

Introduction
The CYP1A1 gene produces a Phase I enzyme, aromatic hydrocarbon hydroxylase, which is responsible for the first oxidative step in the metabolism of many substrates including polycyclic aromatic hydrocarbons like benzo(a)pyrene from tobacco smoke, steroids with a phenol ring, combustion products from meats and fats, and caffeine (1–3). Combined with Phase II enzymes, steroids and xenobiotics are “detoxified” for excretion; however, some of the initial resulting metabolites are highly reactive oxygen species that can damage DNA and potentially promote cancer development. Thus, there has been considerable interest in the relationship between CYP1A1 and cancer, including whether polymorphic variants of the gene may influence cancer risk.

Four CYP1A1 variants have been described, only two of which have been extensively studied in relation to cancer risk (4–6). The Msp1 restriction site polymorphism is found at the 3’ end of the noncoding region of the gene, and Ile/Val is a point mutation at position 4889 in exon 7 resulting in a change from an Ile to Val residue, with Ile as the more common form. CYP1A1 variants have been studied in connection with lung, colon, and breast cancer (6–10), and recently ovarian cancer. Goodman et al. (11) found no association between the CYP1A1 polymorphisms and ovarian cancer overall but did observe that smokers with the Msp1 variant had a greater risk for ovarian cancer than nonsmokers with the variant.

In the study reported here, we sought to examine the relationship between ovarian cancer and the Msp1 and Ile/Val polymorphisms of CYP1A1, and the interaction between these polymorphisms and various exposures, which might either be related to ovarian cancer risk or be affected by the CYP1A1 metabolic pathway (12–21).

Materials and Methods
Subjects, Questionnaires, and Specimens. Details of the study have been published elsewhere (21). Briefly, from May 1992 to March 1997, 1080 incident cases of ovarian cancer were identified through Massachusetts and New Hampshire tumor registries. Age-matched controls were selected through a combination of random digit dialing and town book listings. Demographic information, reproductive and medical history, and habits were assessed by in-person interview at the time of enrollment. A validated self-administered food-frequency questionnaire (22) assessed the frequency of consumption of stated portions of specified foods and beverages. Average daily intakes of nutrients and caffeine were calculated as described previously (12, 23). All of the questions were framed with respect to a reference date defined as 1 year before the diagnosis date for cases and the date of interview for controls.

Heparanized blood was collected at the time of interview, and separated into plasma, red cell, and buffy coat (white cell) components. Some of the buffy coat-enriched specimen was blotted onto filter paper, which we have found to be suitable for direct use in some PCRs including those used in connection with the CYP1A1 polymorphisms tested here. Four hundred forty-five cases and 472 controls had filter paper DNA specimens available for testing.

Genotype Analysis. We performed genotype analysis, blind to case-control status, using PCR then restriction fragment length polymorphism techniques. The following forward and reverse primers were used: for Msp1 polymorphism, 5’-TAGGACTTTCTTCATGCCT-3’ and 5’-CAGTGAA-GAGGTTAGCCGCT-3’, and for Ile/Val polymorphism,
Table 1  Frequency distribution of CYP1A1 polymorphisms (Msp1 and Ile/Val)\(^a\)

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>RR(^a) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Msp1</td>
<td>Wild-type</td>
<td>335</td>
<td>365</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Heterozygous variant</td>
<td>98</td>
<td>94</td>
<td>1.15 (0.83, 1.59)</td>
</tr>
<tr>
<td></td>
<td>Homozygous variant</td>
<td>5</td>
<td>6</td>
<td>0.96 (0.29, 3.24)</td>
</tr>
<tr>
<td></td>
<td>Any variant(^c)</td>
<td>103</td>
<td>100</td>
<td>1.14 (0.83, 1.57)</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>Wild-type</td>
<td>401</td>
<td>434</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Heterozygous variant</td>
<td>37</td>
<td>37</td>
<td>1.09 (0.67, 1.76)</td>
</tr>
<tr>
<td></td>
<td>Homozygous variant</td>
<td>2</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Any variant(^c)</td>
<td>39</td>
<td>37</td>
<td>1.15 (0.71, 1.86)</td>
</tr>
<tr>
<td>Both</td>
<td>Msp1 wild—Ile/Val wild</td>
<td>329</td>
<td>362</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Msp1 wild—Ile/Val variant</td>
<td>1</td>
<td>3</td>
<td>0.29 (0.03, 2.89)</td>
</tr>
<tr>
<td></td>
<td>Msp1 variant—Ile/Val wild</td>
<td>66</td>
<td>65</td>
<td>0.96 (0.66, 1.41)</td>
</tr>
<tr>
<td></td>
<td>Msp1 variant—Ile/Val variant</td>
<td>37</td>
<td>34</td>
<td>1.02 (0.62, 1.68)</td>
</tr>
</tbody>
</table>

\(^a\) For the Msp1 polymorphism 7 case samples and 7 of the control samples were unable to be amplified for genotype analysis. For the Ile/Val polymorphism 5 of the case samples and 1 control sample were unable to be amplified for genotype analysis.

\(^b\) Adjusted for age, site, parity, OC use, family history of breast or ovarian cancer.

\(^c\) Refers to any genotype containing a variant allele.

\(^d\) Since there are no controls with two variant alleles, it was not possible to estimate a RR.

5′-GAACGTCCACCTCCGCTGTCT-3′ and 5′-GAAGACCTCCACGGGCTCA-3′.

PCR products were digested by the Msp1 enzyme to detect the Msp1 polymorphism and the HincII restriction enzyme to detect the Ile/Val polymorphism (24). Of the 917 DNA samples, 6 samples in the Ile/Val analysis and 14 in the Msp1 analysis were not genotyped successfully.

**Statistical Analysis.** A test of the Hardy-Weinberg equilibrium was performed to compare observed with expected genotype frequencies. Unconditional logistic regression was used to determine whether possession of a variant allele is associated with an increased risk of ovarian cancer compared with possession of two wild-type alleles. These analyses were adjusted for age, site, parity, OC\(^2\) use, and family history of breast or ovarian cancer in a first degree relative. On the basis of a priori hypotheses, analyses were performed to assess whether body mass index, OC use, parity, caffeine intake, smoking, analgesic use, and age might modify the effect of CYP1A1 variant genotypes on ovarian cancer risk. Additional exploratory analyses were performed to examine potential effect modification by dietary micro/macronutrients associated with vegetable or meat intake. The statistical significance of effect modification by these variables was tested using the Breslow-Day test of homogeneity. In addition, the effect of variant CYP1A1 genotypes on ovarian cancer risk was assessed by histological subtype of epithelial ovarian cancer.

**Results**

Cases and controls had similar distributions among study center, age group, and body mass index categories. Compared with controls, cases were more likely to be nulliparous, to have a family history of breast or ovarian cancer, and less likely to have used OCs for at least 3 months (data not shown).

The distribution of CYP1A1 polymorphic variants in cases and controls was not significantly different (Table 1). The observed distribution of genotypes was equal to the expected distribution under the assumption of Hardy-Weinberg equilibrium. Overall, possession of an Msp1 variant either in the heterozygous or homozygous state was associated with an adjusted RR (and 95% CI) of 1.14 (0.83–1.57). Overall, possession of an Ile/Val variant either in the heterozygous or homozygous state was associated with an adjusted RR (and 95% CI) of 1.15 (0.71–1.86). However, these data also demonstrate linkage disequilibrium between Msp1 and Ile/Val, because women who were heterozygous or homozygous for the Ile/Val variant almost always possessed the Msp1 variant as well (\(P < 0.001\)).

For both the Msp1 and Ile/Val variants, no significant interactions were noted with age, parity, OC use, analgesic use, or smoking. A significantly elevated risk was seen in women who possessed an Ile/Val variant and who consumed more than the median of 204.5 mg/day of caffeine (RR = 2.69; 95% CI, 1.18–6.18). An exploratory review of dietary variables revealed significantly elevated risks in women who possessed an Ile/Val variant and consumed more than the median of 34.1 g/day of animal fat (RR = 2.19; 95% CI, 1.06–4.64). The interactions involving these variables were statistically significant (\(P = 0.01\) for the caffeine and \(P = 0.04\) for the animal fat).

We also evaluated the frequency of the Msp1 and Ile/Val CYP1A1 polymorphisms within histological categories of epithelial ovarian cancer. The relationship between CYP1A1 polymorphisms and ovarian cancer did not vary significantly by the histological types (data not shown).

**Discussion**

Although we found no overall association between these two CYP1A1 polymorphisms and ovarian cancer risk, we did observe interaction between the Ile/Val polymorphism and caffeine, as well as animal fat. In interpreting these findings, it is useful to have a broader understanding of the role of CYP1A1 in carcinogen activation or detoxification, the possible biological basis for some of the interactions observed, and the limitations of this study.

A premise of our study is that the activity of CYP1A1 may be determined by polymorphic variants. Significant interactions were observed only with the Ile/Val polymorphism, suggesting this polymorphism may have greater biological consequences. However, it should be noted and has been described previously that the Ile/Val variant occurs almost exclusively among those possessing the Msp1 variant (6). Whereas this may pose a...
problem disentangling effects in epidemiological studies, experimental studies may be informative. When DNA encoding the aryl hydrocarbon receptor, other mono-oxygenases like CYP1A1 but other monooxygenases, such as CYP1A2. Thus, understanding of the role of CYP1A1 in determining cancer risk may require examination of not only CYP1A1 polymorphisms, but also those which might affect aryl hydrocarbon receptor, other mono-oxygenases like CYP1A2, and the Phase II enzymes like N-acetyltransferase 2 and xanthine oxidase needed for complete detoxification of steroids and xenobiotics. In a study of lung cancer in a Japanese population, the Ile/Val variant of CYP1A1 was examined in combination with the GSTM1 null polymorphism and was associated with an odds ratio of 27 (24).

Despite the limited scope of our investigation, some potentially interesting interactions were observed in this study including those with dietary caffeine and animal fat. The relationship between caffeine and ovarian cancer independent of genetic polymorphisms has been inconclusive. Most studies showed positive but generally nonsignificant associations (12–16). Previously, in our study population, we found a significant increase in cancer risk with caffeine consumption, but only in premenopausal women (12). The analysis presented in the current paper suggests that the relationship between caffeine and ovarian cancer depends on whether a woman carries a wild-type or variant CYP1A1 Ile/Val allele. The interaction between CYP1A1 polymorphisms and caffeine may explain why the relationship between caffeine and ovarian cancer has been unclear in the past, because the effect of caffeine is diluted when the CYP1A1 genotypes are evaluated together. Similar controversy exists regarding whether fat (20) might increase risk for ovarian cancer, and again our data suggests that this discrepancy may be because of genetic variation.

Much previous research on CYP1A1 and cancer has focused on lung cancer susceptibility because CYP1A1 metabolizes benzo(a)pyrene, a polycyclic aromatic hydrocarbon found in tobacco smoke. The interaction between smoking and the Msp1 variant of CYP1A1 has also been examined with regard to ovarian cancer risk. Although no association was evident between CYP1A1 and ovarian cancer alone, Goodman et al. (11) did find an increased risk of ovarian cancer in smokers with the Msp1 variant compared with smokers with two wild-type alle-
Limitations of our study include issues of power and biases. Because the prevalence of the Ile/Val variant is low in our population, issues of power affect even a relatively large study such as this, particularly when subjects are divided into subgroups to evaluate effect modification. In our population, 8% of women possess the Ile/Val variant, and 22% possess the Msp1 variant. At a significance level of 0.05 and power of 0.8, we had the ability to detect a relative risk of 1.4 for the Msp1 polymorphism and a relative risk of 1.75 for the Ile/Val polymorphism. It should be appreciated that most studies linking CYP1A1 with cancer are conducted in study populations (e.g., the Japanese) that have a higher prevalence of the Ile/Val variant (up to 30%; Ref. 25).

Regarding the issue of bias, our study is susceptible to many of the problems of case-control studies. Cases may recall past exposures differently than controls. However, caffeine intake and dietary factors are not well-known risk factors for ovarian cancer, and, therefore, self-report is unlikely to differ by disease status. If CYP1A1 genotype is related to survival after diagnosis, then cases who possess the variant genotype will be more likely to be enrolled in the study than those who do not. Therefore, the cases would not represent the genotype distribution in the underlying case population, and there may appear to be an association when, in fact, there is none.

In summary, we found that variation of CYP1A1 genotypes is not predictive of ovarian cancer risk alone. However, this gene may alter the effect of certain dietary factors, particularly caffeine, on ovarian cancer risk. Confirmation of this association is needed in other populations, and interaction with other genetic polymorphisms should be assessed because other metabolic pathways may be involved. Because these polymorphisms are not common, even larger studies will be needed to elucidate genetically determined susceptibility to environmental exposures and the biological pathways of ovarian cancer development.

References
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